

**ABSTRACT**

The present invention is directed to the field of HIV resistance to RT inhibitors and methods of determining the levels and mechanisms of action of HIV resistance. The methods of the present invention may be accomplished using a novel *in vitro* assay that provides a reaction well comprising a template for an HIV RT enzyme, a primer, a detectable dNTP substrate, an HIV RT inhibitor, and a ribonucleotide chosen from ATP and GTP or a pyrophosphate. The RT activity is determined by measuring the amount of the detectable dNTP substrate that is incorporated into the template, and the level and/or mechanism of resistance of HIV to the HIV RT inhibitor is determined using the RT activity. The methods may also be used for designing new therapies, screening for new drugs and treatments, and determining the role of mutations in observed resistance.